Claims

1. A method for diagnosing a disorder characterized by expression of MAGE-A1 in a subject typed as HLA-B35 positive, comprising:

contacting a biological sample isolated from the subject with an agent that is specific for a MAGE-A1 HLA binding peptide which comprises SEQ ID NO:10, and

determining the interaction between the agent and the MAGE-A1 HLA binding peptide as a determination of the disorder.

2. The method of claim 1 wherein the MAGE-A1 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8, and (iii) functional variants of the peptides of (i) and (ii).

3. A method for diagnosing a disorder characterized by expression of MAGE-A1 in a subject typed as HLA-B44 positive, comprising:

contacting a biological sample isolated from the subject with an agent that is specific for a MAGE-A1 HLA binding peptide which comprises SEQ ID NO:53, and

determining the interaction between the agent and the MAGE-A1 HLA binding peptide as a determination of the disorder.

4. The method of claim 3 wherein the MAGE-A1 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14, and (iii) functional variants of the peptides of (i) and (ii).

5. A method for diagnosing a disorder characterized by expression of a complex of a MAGE-A1 HLA-B35 or HLA-B44 binding peptide and a HLA-B35 or HLA-B44 molecule, comprising:

contacting a biological sample isolated from a subject suspected of having the disorder with an agent that binds the complex; and

determining binding between the complex and the agent as a determination of the disorder.

6. The method of claim 5 wherein the MAGE-A1 HLA-B35 binding peptide is selected from the group consisting (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:10, and (iii) functional variants of the peptides of (i) and (ii).

7. The method of claim 5 wherein the MAGE-A1 HLA-B44 binding peptide is selected from the group consisting (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:53, and (iii) functional variants of the peptides of (i) and (ii).

8. A method for enriching selectively a population of T lymphocytes with T lymphocytes specific for a MAGE-A1 HLA binding peptide, comprising:

contacting a source of T lymphocytes which contains a population of T lymphocytes with an agent presenting a complex of a MAGE-A1 HLA-B35 binding peptide comprising SEQ ID NO:10 and a HLA-B35 molecule or a complex of a MAGE-A1 HLA-B44 binding peptide comprising SEQ ID NO:53 and a HLA-B44 molecule, in an amount sufficient to selectively enrich the population of T lymphocytes with the T lymphocytes specific for one of the complexes.

9. The method of claim 8, wherein the agent is selected from the group consisting of an antigen presenting cell which expresses a HLA-B35 molecule contacted with a MAGE-A1 protein or a HLA binding fragment thereof which comprises SEQ ID NO:10, and an antigen presenting cell which expresses a HLA-B44 molecule contacted with a MAGE-A1 protein or a HLA binding fragment thereof which comprises SEQ ID NO:53.

10. The method of claim 8 wherein the MAGE-A1 HLA-B35 binding peptide is selected from the group consisting of (i) SEQ ID NO:8, SEQ ID NO:9 and SEQ ID NO:10, and (ii) functional variants of the peptides of (i).

11. The method of claim 8 wherein the MAGE-A1 HLA-B44 binding peptide is selected from the group consisting of (i) SEQ ID NO:12 and SEQ ID NO:14, and (ii) functional

variants of the peptides of (i).

12. A method for treating a subject, typed as HLA-B35 or HLA-B44 positive, having a disorder characterized by expression of MAGE-A1, comprising:

administering to the subject an amount of a MAGE-A1 HLA-B35 binding peptide comprising SEQ ID NO:10 or a MAGE-A1 HLA-B44 binding peptide comprising SEQ ID NO:53 sufficient to ameliorate the disorder.

13. The method of claim 12, wherein the MAGE-A1 HLA-B35 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8, and (iii) functional variants of the peptides of (i) and (ii).

14. The method of claim 13, further comprising administering to the subject at least one isolated HLA binding peptide selected from the group consisting of (1) MAGE-A1 HLA class I binding peptides other than peptides comprising SEQ ID NO:8, (2) MAGE-A1 HLA class II binding peptides, and (3) MAGE-A1 HLA class I or class II binding peptide of a non-MAGE-A1 tumor antigen, in an amount sufficient to ameliorate the disorder.

15. The method of claim 12, wherein the MAGE-A1 HLA-B44 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14, and (iii) functional variants of the peptides of (i) and (ii).

16. The method of claim 15, further comprising administering to the subject at least one isolated HLA binding peptide selected from the group consisting of (1) MAGE-A1 HLA class I binding peptides other than peptides comprising SEQ ID NO:14, (2) MAGE-A1 HLA class II binding peptides, and (3) MAGE-A1 HLA class I or class II binding peptide of a non-MAGE-A1 tumor antigen, in an amount sufficient to ameliorate the disorder.

17. A method for treating a subject, typed as HLA-B35 or HLA-B44 positive, having a disorder characterized by expression of MAGE-A1, comprising:

administering to the subject an amount of an agent which enriches selectively in the subject the presence of complexes of a HLA-B35 molecule and a MAGE-A1 HLA-B35 binding peptide comprising SEQ ID NO:10, or a HLA-B44 molecule and a MAGE-A1 HLA-B44 binding peptide comprising SEQ ID NO:53, in an amount sufficient to ameliorate the disorder.

18. The method of claim 17, wherein the agent comprises a MAGE-A1 HLA-B35 binding peptide selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8, and (iii) functional variants of the peptides of (i) and (ii).

19. The method of claim 17, wherein the agent comprises a MAGE-A1 HLA-B44 binding peptide selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14, and (iii) functional variants of the peptides of (i) and (ii).

20. A method for treating a subject, typed as HLA-B35 or HLA-B44 positive, having a disorder characterized by expression of MAGE-A1, comprising:

administering to the subject an amount of autologous T lymphocytes sufficient to ameliorate the disorder, wherein the T lymphocytes are specific for complexes of a HLA-B35 molecule and a MAGE-A1 HLA-B35 binding peptide comprising SEQ ID NO:10, or a HLA-B44 molecule and a MAGE-A1 HLA-B44 binding peptide comprising SEQ ID NO:53.

21. The method of claim 20 wherein the MAGE-A1 HLA-B35 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8, and (iii) functional variants of the peptides of (i) and (ii).

22. The method of claim 20 wherein the MAGE-A1 HLA-B44 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14, and (iii) functional variants of the peptides of (i) and (ii).

23. A method for identifying functional variants of a MAGE-A1 HLA-B35 or HLA-B44 binding peptide, comprising

providing (1) a MAGE-A1 HLA-B35 binding peptide comprising SEQ ID NO:10, and a HLA-B35 molecule, or (2) a MAGE-A1 HLA-B44 binding peptide comprising SEQ ID NO:53, and a HLA-B44 molecule, and a T cell which is stimulated by the MAGE-A1 HLA binding peptide presented by the HLA-B35 or HLA-B44 molecule;

mutating a first amino acid residue of the MAGE-A1 HLA-B35 or HLA-B44 binding peptide to prepare a variant peptide; and

determining the binding of the variant peptide to the HLA-B35 or HLA-B44 molecule or the stimulation of the T cell, wherein binding of the variant peptide to the HLA binding molecule or stimulation of the T cell by the variant peptide presented by the HLA binding molecule indicates that the variant peptide is a functional variant.

24. The method of claim 23, wherein the MAGE-A1 HLA-B35 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8.

25. The method of claim 23, wherein the MAGE-A1 HLA-B44 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14.

26. The method of claim 23, further comprising the step of comparing the stimulation of the T cell by the MAGE-A1 HLA binding peptide and the stimulation of the T cell by the functional variant as a determination of the effectiveness of the stimulation of the T cell by the functional variant.

27. An expression vector comprising a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, operably linked to a promoter, and a nucleotide sequence which encodes the amino

acid sequence of a HLA-B*35 molecule, operably linked to a promoter.

28. An expression vector comprising a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:53, operably linked to a promoter, and a nucleotide sequence which encodes the amino acid sequence of a HLA-B*44 molecule operably linked to a promoter.

29. A host cell transfected or transformed with the expression vector of claim 27.

30. A host cell transfected or transformed with the expression vector of claim 28.

31. An isolated T lymphocyte which selectively binds a complex of a HLA-B35 molecule and a MAGE-A1 HLA binding peptide which comprises the amino acid sequence of SEQ ID NO:10.

32. The isolated T lymphocyte of claim 31 wherein the MAGE-A1 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8, and (iii) functional variants of the peptides of (i) and (ii).

33. An isolated T lymphocyte which selectively binds a complex of a HLA-B44 molecule and a MAGE-A1 HLA binding peptide which comprises the amino acid sequence of SEQ ID NO:53.

34. The isolated T lymphocyte of claim 33 wherein the MAGE-A1 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14, and (iii) functional variants of the peptides of (i) and (ii).

35. An isolated antigen presenting cell which comprises a complex of a HLA-B35 molecule and a MAGE-A1 HLA binding peptide which comprises the amino acid sequence of SEQ ID NO:10.

36. The isolated antigen presenting cell of claim 35 wherein the MAGE-A1 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:8, and (iii) functional variants of the peptides of (i) and (ii).

37. An isolated antigen presenting cell which comprises a complex of a HLA-B44 molecule and a MAGE-A1 HLA binding peptide which comprises the amino acid sequence of SEQ ID NO:53.

38. The isolated antigen presenting cell of claim 37 wherein the MAGE-A1 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:2, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:14, and (iii) functional variants of the peptides of (i) and (ii).

39. A vaccine composition comprising a cell selected from the group consisting of a T lymphocyte of claims 31-34 and an antigen presenting cell of claims 35-38, and a pharmaceutically acceptable carrier.

40. The vaccine composition of claim 39, further comprising an adjuvant.

41. An isolated functional variant of a MAGE-A1 HLA-B35 or HLA-B44 binding peptide identified by the method of claim 23.

42. A method for identifying a candidate mimetic of a MAGE-A1 HLA-B35 or HLA-B44 binding peptide, comprising

providing a HLA-B35 or HLA-B44 molecule,

contacting the HLA molecule with a test molecule, and

determining the binding of the test molecule to the HLA molecule, wherein a test molecule which binds to the HLA molecule is a candidate mimetic of the MAGE-A1 HLA binding peptide.

43. The method of claim 42, further comprising contacting the HLA molecule with a HLA-B35 binding molecule comprising the amino acid sequence of SEQ ID NO:8, and determining the binding of the HLA binding molecule to the HLA molecule in the presence and the absence of the test molecule.

44. The method of claim 42, further comprising contacting the HLA molecule with a HLA-B44 binding molecule comprising the amino acid sequence of SEQ ID NO:53, and determining the binding of the HLA binding molecule to the HLA molecule in the presence and the absence of the test molecule.

45. The method of claim 42, further comprising

forming a complex of the HLA molecule and the candidate mimetic,

contacting the complex with a T cell which binds to a complex of a HLA molecule and the MAGE-A1 HLA binding peptide, and

assaying activation of the T cell.

46. The method of claim 45, wherein activation of the T cell is indicated by a property selected from the group consisting of proliferation of the T cell, interferon-γ production by the T cell, tumor necrosis factor production by the T cell, and cytolysis of a target cell by the T cell.

47. An isolated mimetic of a MAGE-A1 HLA-B35 or HLA-B44 binding peptide identified by the method of claim 42.

48. A method for diagnosing a disorder characterized by expression of MAGE-A3 in a subject typed as HLA-B35 positive, comprising:

contacting a biological sample isolated from the subject with an agent that is specific for a MAGE-A3 HLA binding peptide which comprises SEQ ID NO:56, and

determining the interaction between the agent and the MAGE-A3 HLA binding peptide as a determination of the disorder.

49. The method of claim 48 wherein the MAGE-A3 HLA binding peptide is selected from

the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59, and (iii) functional variants of the peptides of (i) and (ii).

50. A method for diagnosing a disorder characterized by expression of a complex of a MAGE-A3 HLA-B35 binding peptide and a HLA-B35 molecule, comprising:

contacting a biological sample isolated from a subject suspected of having the disorder with an agent that binds the complex; and

determining binding between the complex and the agent as a determination of the disorder.

51. The method of claim 50 wherein the MAGE-A3 HLA-B35 binding peptide is selected from the group consisting (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:56, and (iii) functional variants of the peptides of (i) and (ii).

52. A method for enriching selectively a population of T lymphocytes with T lymphocytes specific for a MAGE-A3 HLA binding peptide, comprising:

contacting a source of T lymphocytes which contains a population of T lymphocytes with an agent presenting a complex of a MAGE-A3 HLA-B35 binding peptide comprising SEQ ID NO:56 and a HLA-B35 molecule, in an amount sufficient to selectively enrich the population of T lymphocytes with the T lymphocytes specific for one of the complexes.

53. The method of claim 52, wherein the agent is an antigen presenting cell which expresses a HLA-B35 molecule contacted with a MAGE-A3 protein or a HLA binding fragment thereof which comprises SEQ ID NO:56.

54. The method of claim 52 wherein the MAGE-A3 HLA-B35 binding peptide is selected from the group consisting of (i) SEQ ID NO:56, SEQ ID NO:57 and SEQ ID NO:59, and (ii) functional variants of the peptides of (i).

55. A method for treating a subject, typed as HLA-B35 positive, having a disorder

characterized by expression of MAGE-A3, comprising:

administering to the subject an amount of a MAGE-A3 HLA-B35 binding peptide comprising SEQ ID NO:56 sufficient to ameliorate the disorder.

56. The method of claim 55, wherein the MAGE-A3 HLA-B35 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59, and (iii) functional variants of the peptides of (i) and (ii).

57. The method of claim 55, further comprising administering to the subject at least one isolated HLA binding peptide selected from the group consisting of (1) MAGE-A3 HLA class I binding peptides other than peptides comprising SEQ ID NO:59, (2) MAGE-A3 HLA class II binding peptides, and (3) HLA class I or class II binding peptide of a non-MAGE-A3 tumor antigen, in an amount sufficient to ameliorate the disorder.

58. A method for treating a subject, typed as HLA-B35 positive, having a disorder characterized by expression of MAGE-A3, comprising:

administering to the subject an amount of an agent which enriches selectively in the subject the presence of complexes of a HLA-B35 molecule and a MAGE-A3 HLA-B35 binding peptide comprising SEQ ID NO:56, in an amount sufficient to ameliorate the disorder.

59. The method of claim 58, wherein the agent comprises a MAGE-A3 HLA-B35 binding peptide selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59, and (iii) functional variants of the peptides of (i) and (ii).

60. A method for treating a subject, typed as HLA-B35 positive, having a disorder characterized by expression of MAGE-A3, comprising:

administering to the subject an amount of autologous T lymphocytes sufficient to ameliorate the disorder, wherein the T lymphocytes are specific for complexes of a HLA-B35 molecule and a MAGE-A3 HLA-B35 binding peptide comprising SEQ ID NO:56.

61. The method of claim 60 wherein the MAGE-A3 HLA-B35 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59, and (iii) functional variants of the peptides of (i) and (ii).

62. A method for identifying functional variants of a MAGE-A3 HLA-B35 binding peptide, comprising

providing a MAGE-A3 HLA-B35 binding peptide comprising SEQ ID NO:56, a HLA-B35 molecule, and a T cell which is stimulated by the MAGE-A3 HLA binding peptide presented by the HLA-B35 molecule;

mutating a first amino acid residue of the MAGE-A3 HLA-B35 binding peptide to prepare a variant peptide; and

determining the binding of the variant peptide to the HLA-B35 molecule or the stimulation of the T cell, wherein binding of the variant peptide to the HLA binding molecule or stimulation of the T cell by the variant peptide presented by the HLA binding molecule indicates that the variant peptide is a functional variant.

63. The method of claim 62, wherein the MAGE-A3 HLA-B35 binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59.

64. The method of claim 62, further comprising the step of comparing the stimulation of the T cell by the MAGE-A3 HLA binding peptide and the stimulation of the T cell by the functional variant as a determination of the effectiveness of the stimulation of the T cell by the functional variant.

65. An expression vector comprising a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:56, SEQ ID NO:57 and SEQ ID NO:59, operably linked to a promoter, and a nucleotide sequence which encodes the amino acid sequence of a HLA-B35 molecule, operably linked to a promoter.

66. A host cell transfected or transformed with the expression vector of claim 65.

67. An isolated T lymphocyte which selectively binds a complex of a HLA-B35 molecule and a MAGE-A3 HLA binding peptide which comprises the amino acid sequence of SEQ ID NO:56.

68. The isolated T lymphocyte of claim 67 wherein the MAGE-A3 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59, and (iii) functional variants of the peptides of (i) and (ii).

69. An isolated antigen presenting cell which comprises a complex of a HLA-B35 molecule and a MAGE-A3 HLA binding peptide which comprises the amino acid sequence of SEQ ID NO:56.

70. The isolated antigen presenting cell of claim 69 wherein the MAGE-A3 HLA binding peptide is selected from the group consisting of (i) peptides which consist of a fragment of the amino acid sequence of SEQ ID NO:55, (ii) peptides which comprise the amino acid sequence of SEQ ID NO:59, and (iii) functional variants of the peptides of (i) and (ii).

71. A vaccine composition comprising a cell selected from the group consisting of a T lymphocyte of claims 67-68 and an antigen presenting cell of claims 69-70, and a pharmaceutically acceptable carrier.

72. The vaccine composition of claim 71, further comprising an adjuvant.

73. An isolated functional variant of a MAGE-A3 HLA-B35 binding peptide identified by the method of claim 62.

74. A method for identifying a candidate mimetic of a MAGE-A3 HLA-B35 binding peptide, comprising

providing a HLA-B35 molecule,

contacting the HLA molecule with a test molecule, and

determining the binding of the test molecule to the HLA molecule, wherein a test molecule which binds to the HLA molecule is a candidate mimetic of the MAGE-A3 HLA binding peptide.

75. The method of claim 74, further comprising contacting the HLA molecule with a HLA-B35 binding molecule comprising the amino acid sequence of SEQ ID NO:56, and determining the binding of the HLA binding molecule to the HLA molecule in the presence and the absence of the test molecule.

76. The method of claim 74, further comprising

forming a complex of the HLA molecule and the candidate mimetic,

contacting the complex with a T cell which binds to a complex of a HLA molecule and the MAGE-A3 HLA binding peptide, and

assaying activation of the T cell.

77. The method of claim 76, wherein activation of the T cell is indicated by a property selected from the group consisting of proliferation of the T cell, interferon-γ production by the T cell, tumor necrosis factor production by the T cell, and cytolysis of a target cell by the T cell.

78. An isolated mimetic of a MAGE-A3 HLA-B35 binding peptide identified by the method of claim 74.